

ANALYSIS OF MASS SPECTRAL DATA OF DITERPENES

ISOLATED FROM *CLERODENDRON INFORTUNATUM*

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ABSTRACT

Terpenes are the largest group of phytochemicals that exhibit beneficial interactions in organisms. Diterpenes of plant are dominated in research area due to their novel chemical structure and medicinal values. The majority of diterpenes exhibited cytotoxic, antitumor and antimicrobial activities in vitro. Several diterpenes are reported in Clerodendron infortunatum. The objectives of present study was the isolation, purification and structural investigation of isolated diterpens by compairing ¹H and ¹³C of Mass spectral data and previously known standard structures.

KEYWORDS: Mass Spectral data, Diterpenes, Clerodendron Infortunatum, Clerodin

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INTRODUCTION

Natural products are produced by plants of high and low molecular weight, known as primary and secondary metabolites (Croteau R et.al. 2000). Secondary metabolites have great significance in the field of medicine, agriculture and industries, which attract scientists for research in various biological activities. Plant secondary metabolites contain three types of compounds i.e. Terpenoid, Alkaloid and Phenolic. Terpenoids are largest phytochemical compounds that inhibit various interactions in organisms (Croteau R et.al. 2000). Terpenes are essential for all living organisms for metabolic control and intra species interactions, for example pollination. Plants manufacture terpenoides to attract pollinating insects and also attract beneficial mites, which feed herbivorous insects (Zwenger S et.al. 2008 Kappers IF et.al. 2005). It has been reported that terpene acts as toxins and growth inhibitors in animals and microbes (Croteau R et.al. 2000). Terpenes also act as chemical messengers, who induces gene expression and also influence defensive functions (Cheng et. al., 2007).

Terpenes are biosynthesize by two different pathways, the mevalonic acid pathway (MVA) or the deoxyxylulose phosphate pathway (Eisenreich *et al.* 2001). Diterpenes are found in many plant, have biological activity. Some biologically active diterpenes include taxol, cafestol and kahweol. Diterpenes, isolated from *Taxus brevifolia* and *Coffea arabica*, display anticancer properties (koepp et al 1995). One type of structural class of terpenes are clerodanes (Diterpenes) which are found in many different plant families and contain four contiguous stereocenters contained in a cis or trans decalin. Various clerodane diterpenes have been isolated. These include columbin, isolated from the roots of *Calumbae radix*, which shows anti-cancer chemo-preventive properties, and clerocidin, isolated from *Oidiodendron truncatum*, which has shown antibiotic activity (De la torre et al., 1986).

Clerodendron infortunatum Gaertn (Verbenaceae) is an important medicinal plant, commonly known as Bhand. *Clerodendron infortunatum* is a common shrub of 2 -4m height of the family Verbeaceae, found along road

side in north India and elsewhere, and flowering during February- May (Haines,1925) identified by Linnaeus in 1753. Known in ayurveda by the Sanskrit names “Bharhi”, “Bhrigubhava”, “Padma”, “Fanji”, and “Brahman yastika” (Shastri,1977), as “Peruvelum” in Kerala. The plant is reported to contain active bitter substances like clerodin, which has been widely used as tonic and anti-helminthic agents in the country side of North India. Though, variously used in Ayurveda, Unani system of medicine and Homeopathy in case of ailments like diarrhoea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as vermifuge, laxative and cholagogue, for the removal of ascarids in anus, as external applications on tumours, etc. (Kirtikar and Basu, 1991). Leaves are used as a bitter tonic, vermifuge, laxative and cholagogue. Fresh juice is introduced in the rectum for the removal of ascarids. The plant extract showed its activity against microorganisms (Kumar, 2007). Plant possess antioxidant (Wong et al., 2009), antibacterial (Nair et.al. 2005), antifungal (Khan Wassilew, 1987) and radioprotective (Jagetia et.al. 2005). Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2, -(3, 4-dehydroxyphenyl) ethanol 1-O- α -2 rhamnopyranosyl-(1 \rightarrow 3)- β -D- (4-O-caffeoyl) glycopyranoside or acteoside (Akihisa *et al.* 1989; Sinha *et al.*, 1981). Some reports indicated hepatoprotective, anti-inflammatory, antinociceptive, and neuropharmacological activities of *C. Infortunatum* (Ahmad *et al.*, 2007; Das *et al.*, 2010). The plant requires thorough investigation for its specific medicinally active principles.

Therefore, the investigation of diterpenes in plant *Clerodendron infortunatum* is necessary and present study designed to isolate and evaluate the molecular mass of diterpenes result by HPLC LC-MS.

MATERIALS AND METHODS

Collection of Plant Material and Extraction

Plant was collected from the road side. Leaves of *Clerodendron infortunatum* plants were separated, washed with tap water, rinsed with distilled water, air dried for 1 hour, and then shade dried. They were ground in to powder (coarsely) and stored in room temperature. The extract of the samples were prepared by soaking 2.5kg of dried powder in 3000ml of n-hexane for 72 hours. The extracts were decanted carefully.

Filterate was evaporated under refrigerator and residue was further partitioned with hexane and methanol 1:1. Methanol fraction was collected and kept under refrigerator for crystallization. Crystals were separated using spatula carefully, weigh and kept in the refrigerator for further use. 10gm crystal were subjected to silicagel column and eluted with hexane containing increasing amount of ethyl acetate.

Following ratio of mobile phase was used for fractionation;

Table1

Fraction no.1	Hexane: Ethyl acetate	100: 00
Fraction no.2	Hexane: Ethyl acetate	95: 05
Fraction no.3	Hexane: Ethyl acetate	90: 10
Fraction no.4	Hexane: Ethyl acetate	85: 15
Fraction no.5	Hexane: Ethyl acetate	80: 20
Fraction no.6	Hexane: Ethyl acetate	75: 25
Fraction no.7	Hexane: Ethyl acetate	70: 30
Fraction no.8	Hexane: Ethyl acetate	65: 35
Fraction no.9	Hexane: Ethyl acetate	60: 40
Fraction no.10	Hexane: Ethyl acetate	55: 45

MONITORING OF FRACTIONS

Fractions were monitored by TLC plate of 0.25mm thick. For TLC Chloroform and methanol 97:3 was used for mobile phase. Spots were visualized by spray with 20% H_2SO_4 under UV ray. Sixth fraction was showed spot on TLC plate, so this fraction was sent to SAIF CDRI for HPLC LC-MS.

RESULT AND DISCUSSIONS

Liquid chromatographic result of fraction 6 Hexane: ethyl acetate 75:25 is shown in graph. Absorption maxima at wave length 209 nm in 13.30 minutes were observed in HPLC result. LC and MS graphs of isolated molecules are compared with standard graph of previously known diterpenes found in *Clerodendron infortunatum* by fractionation method.

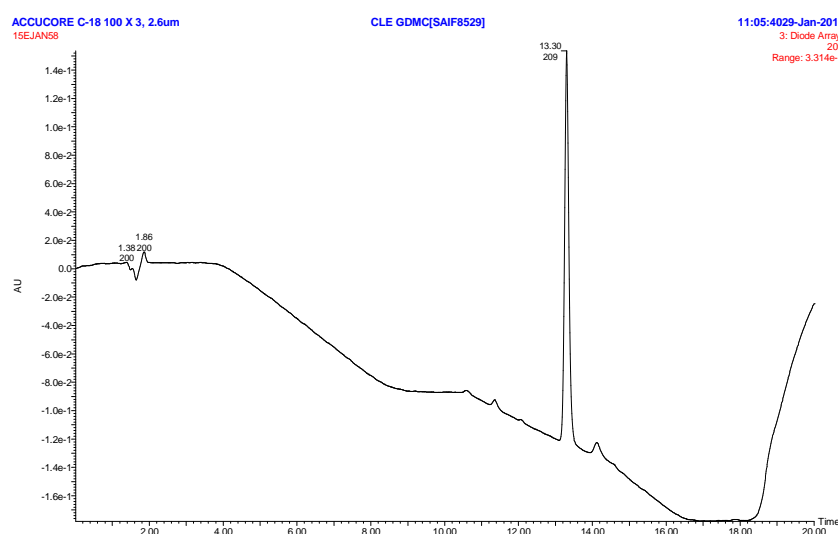


Figure 1: LC Graph

Table 2

Molecular Mass	Molecular Formula	Retention Time	Principal Peaks
476	$\text{C}_{25} \text{H}_{48} \text{O}_8$	13.29	113 (Base peak), 159, 177, 187, 205, 229, 259, 279, 297, 315, 333, 377, 393, 415, 437, 454, 459, 476
475	$\text{C}_{25} \text{H}_{47} \text{O}_8$	11.33	113, 161, 177, 187, 205, 215, 233, 251, 259, 279, 297, 315, 333, 367, 375, 393 (Base peak), 415, 437, 454, 460, 475
475	$\text{C}_{25} \text{H}_{47} \text{O}_8$	11.33	113, 161, 177, 187, 205, 215, 233, 251, 259, 279, 297, 315, 333, 367, 375, 393 (Base Peak), 415, 437, 454, 459, 475
473	$\text{C}_{25} \text{H}_{45} \text{O}_8$	11.22	111, 147, 177, 187, 205 (Base peak), 215, 233, 251, 255, 269, 279, 297, 315, 367, 375, 435, 452, 458, 473.

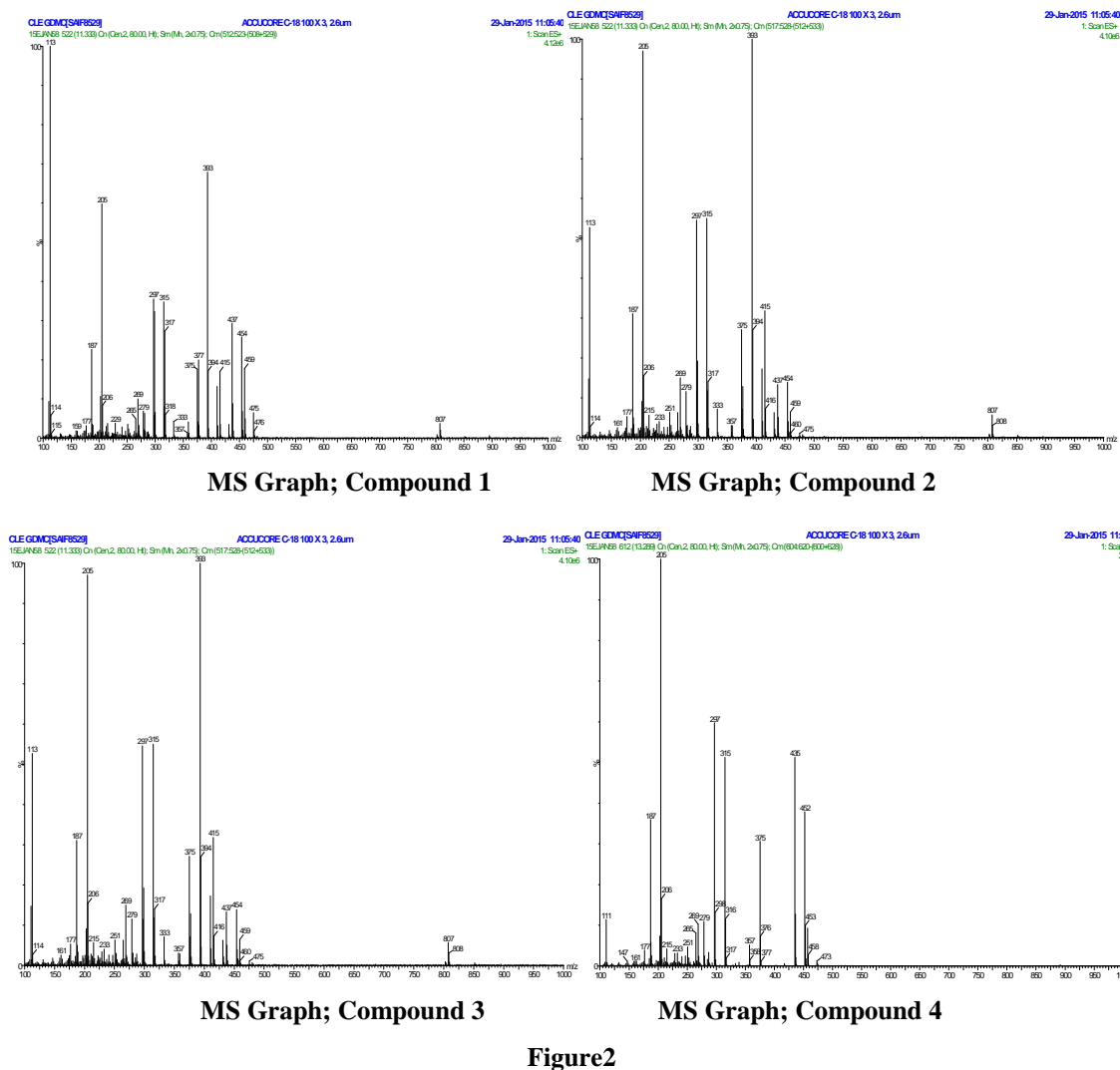


Figure2

Mass spectral data shows four identical compounds were isolated in Hexane: Ethyl acetate 75: 25 (Fraction no. 6). Compound 1 possesses molecular formula $C_{25}H_{48}O_8$ of molecular weight 476 and retention time 13.29 min. Compound 2 and 3 of same molecular weight 475 and formula $C_{25}H_{47}O_8$ and retention time 11.33min, whereas compound 4 has slightly different molecular weight 473 and formula $C_{25}H_{45}O_8$. More than 20 carbon compounds confirm the isolated molecule is Diterpene. Base peak of compound 1 is at m/z 113 due to furofuran ring with (2 protons) forms by the cleavage of C-11, but in compound 2 and 3 base peak is at m/z 393 and significant peak is at m/z 113 due to furofuran ring with two protons. Base peak of compound 4 is m/z 205 and significant peak at m/z 111 with no extra protons confirms the presence of furofuran ring in isolated molecule.

The entire four compounds show a peak at m/z 315 due to breakage of C-10, confirms the presence of Decalin ring. First three compounds have a base peak at m/z 393 due to loss of acetate group at C-5. All compounds have an MS peak at m/z 187 and 297 loss of water molecule gives a peak at 279 due to the breakage of C-5 and C-6 acetate. Loss of acetate group from C-7 gives a peak at m/z 415. Compound 4 shows a peak at m/z 177, 259 and 298 due to break of C-6 and C-7. Due to loss of acetate group from C-7 position measured peak at m/z 415. All peaks are compared with previously known standard molecular structure of diterpene Clerodin found in *Clerodendron infortunatum* by fragmentation method.

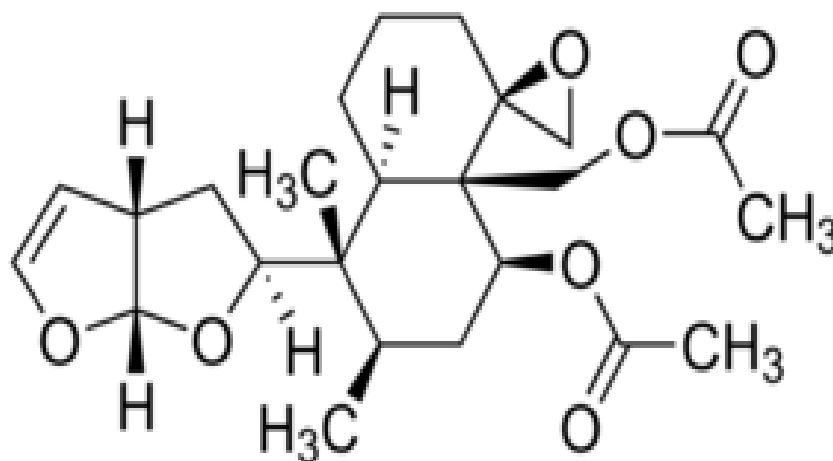


Figure 3: Structure of Clerodin

CONCLUSIONS

It has been concluded that the isolated molecule of fraction 75:25 is diterpene and its molecular formula $C_{25}H_{48}O_8$, $C_{25}H_{47}O_8$, $C_{25}H_{45}O_8$. Structures are more similar to diterpene clerodin, which found in *Clerodendron infortunatum* so, they are derivatives of Clerodin. This analysis revealed the presence of peaks in the mass spectra compatible with the fragmentation pattern of clerodin.

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